REMARKS

I. Support for the Amendments

Support for claims 1, 13-15, 21, 22, 24, 30, 31, 34, 35, and 37 as amended and for new claims 39-44 can be found in the original specification, e.g., from pages 2-5, in the Examples and in the original and amended claims. Detailed support for amended claims 1, 13-15, 21, 22, 24, 30, 31, 34, 35, and 37 can be found on pages 2-4 and 6-7 of the specification. Detailed support for amended claim 15 can also be found on pages 4-5 and 8 of the specification. Detailed support for new claims 39-44 can be found on pages 2-4 and 6-7 of the specification.

II. Status of the Claims

Claims 1-38 were previously pending in the present application, with claims 1 and 24 being the independent claims. In the Office Action, mailed 19 November 2002, the Examiner rejected claims 1-12, 15-29, 32, 33, 36, and 38 and objected to claims 13, 14, 30, 31, 34, 35, and 37.

Currently, claims 1-35 and 37-44 are pending in the application, with claims 1, 13, 14, 24, 30, 31, 34, 35, and 37 being the independent claims. Claims 39-44 are new claims. Claim 36 has been cancelled without prejudice.

III. Rejection of Claims 15-22 under 35 U.S.C. § 112, Second Paragraph, Is Accommodated

The Examiner has rejected claims 15-22 under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. In particular, the Examiner has rejected claims 15 and 21, alleging insufficient antecedent basis for a limitation in each of these claims.

Applicants hereby amend claims 15 and 21 in accordance with the Examiner's remarks. Applicants respectfully assert that the Examiner's rejection of claims 15-22 under 35 U.S.C. § 112, second paragraph, has been accommodated and that claims 15-22 are in a condition for allowance. Therefore, Applicants request reconsideration and withdrawal of the rejections made under 35 U.S.C. §112, second paragraph.

IV. Rejection of Claims 1-3, 5, 6, 24, 28, 32, 33, and 38 Under 35 U.S.C. § 102(b) Is Traversed

In the Office Action at pages 2-3 (paragraph 9), the Examiner has rejected claims 1-3, 5, 6, 24, 28, 32, 33, and 38 as being anticipated by JP 03101688. This rejection is respectfully traversed. The Examiner stated that the abstract of JP 03101688 taught an

> aqueous mixture comprising a chaotrope (guanidinium salt) and butanol in a method of isolating plasmid DNA from an aqueous mixture of plasmid DNA and genomic DNA by adding a chaotrope (guanidinium salt) and butanol, then isolating the plasmid. The aqueous solution was at a basic pH. [Office Action, paragraph 9].

Applicants respectfully submit, however, that neither the abstract, nor the remainder of JP 03101688 anticipates claims 1-3, 5, 6, 24, 28, 32, 33, or 38.

Claim 1 currently reads as follows:

> 1 (amended). A method for isolating plasmid DNA from genomic DNA in a DNA containing material which comprises plasmid DNA and genomic DNA, comprising the steps of:
> (i) extracting the plasmid DNA into butanol by mixing the material with butanol, a chaotrope, and water under conditions to denature the genomic DNA and forming an aqueous phase and a butanol phase, wherein the genomic DNA is substantially in the aqueous phase and the plasmid DNA is substantially in the butanol phase; and
> (ii) recovering the plasmid DNA from the butanol.

Claims 2-3, 5, 6, and 38 are dependent on claim 1.

The Examiner has cited the abstract of JP 03101688. The abstract describes a method by which nucleic acids can be separated from samples or fermentation cultures by treating the sample with a protein denaturant (e.g., guanidine salt, urea, etc.) and then with a substance selected from ethanol, propanol, butanol, pentanol, and hexanol for precipitation or removal. An example is given in which a plasmid-transformed *Escherichia coli* culture medium was treated with guanidine-HCl and stirred with isopropanol. The sample was centrifuged, and the precipitate was washed twice with ethanol, dissolved in Tris buffer (pH 8.0) containing 1 mM EDTA and centrifuged again. The resulting supernatant was treated with ethanol and centrifuged to yield DNA.

As noted previously, the abstract of JP 03101688 describes a method for the separation of **nucleic acids** from **samples or cultures** - **not** the separation of **plasmid DNA** from a **DNA containing material** which comprises plasmid DNA and **genomic DNA**, as outlined in claim 1.

Claim 1 of the present application requires the extraction of plasmid DNA into butanol, which is water-immiscible, while genomic DNA in the sample is substantially in the aqueous phase. The specification of the present application states:

> In a preferred arrangement, the organic solvent is capable of **selectively** supporting the **plasmid** DNA with the **exclusion** of **genomic** DNA present in the plasmid DNA containing material. [p. 2, ll. 22-25, emphasis added.]

It also notes:

> In extraction step (i), the DNA containing material is mixed with the reagents under conditions to denature the genomic DNA typically whereby the **plasmid** DNA is partitioned into an **organic phase** and the

> **genomic** DNA is partitioned into an **aqueous phase**. [p. 2, l. 33 - p. 3, l. 1, emphasis added.]

In addition, it states:

> [I]t is thought that differential solubility between plasmid and genomic DNA under denaturing condition may result in plasmid DNA in an undenatured or reversibly denatured state partitioning into the organic phase. In contrast denatured genomic DNA partitions into the aqueous phase. [p. 3, ll. 18-23.]

However, the example in the abstract of JP03101688 describes isolation of a plasmid from an *E. coli* culture medium by treatment with guanidine-HCl stirred with isopropanol (not butanol) and centrifuged to form a **precipitate**. There is **no** discussion of the **separation** of **plasmid** DNA from **genomic** DNA. Likewise, there is no discussion in the specification of JP 03101688, a translation of which is provided, of the separation of different types of DNA. (Applicants respectfully note that the page numbers used in this Amendment discussion refer to the page numbers of the translation of the specification of JP 03101688.) Of the five Practical Examples, only Practical Example 4 (pp. 9-10) uses secondary butyl alcohol, and this example focuses on isolation of DNA from K562 human leukocyte cancer cells, which are not described as containing a plasmid, because they are **not plasmid-containing** cells. The remaining examples, including the *E. coli* example (Practical Example 3) mentioned in the abstract, use either isopropanol (Practical Example 3) or ethanol (Practical Examples 1, 2, and 5).

In addition, Practical Example 4 does not disclose step ii of claim 1 with regard to recovery of the plasmid DNA from the butanol phase. In Practical Example 4, the butanol was used to precipitate DNA. **DNA** was **recovered from the precipitate**, rather than from the solvent, and, again, there is no mention of any plasmid DNA in this Example, because this Example does not use plasmid-containing cells.

According to the methods of the present invention, the **butanol and aqueous phases are immiscible** (see page 3, ll. 18-30), and the **plasmid DNA and genomic DNA partition differently** into these two immiscible phases, such that plasmid DNA is soluble in the organic solvent, but genomic DNA is not. While the present application also provides methods (e.g., claim 15) for precipitating plasmid DNA from the butanol, this precipitation occurs only after the plasmid DNA has been solubilized in the butanol phase and requires addition of a precipitating agent.

JP 03101688 mentions the use of butanol to precipitate DNA, but **does not teach** either the separation of plasmid DNA from genomic DNA (as required by the language of claims 1 and 24) or the solubility of plasmid DNA in butanol. It **does not even remotely suggest** the desirability of either the separation of types of DNA or the solubility of plasmid DNA in butanol, let alone describe a means of achieving either feature.

In addition, claim 1 requires the extraction to take place "under conditions to denature the genomic DNA." While the abstract describes use of a **protein** denaturant, it does **not** address the issue of **genomic DNA** denaturation, much less discuss its significance. A protein denaturant would not necessarily suffice as a denaturant for genomic DNA.

Likewise, the JP 03101688 specification does not discuss denaturation of genomic DNA. Instead the specification focuses on the concentration limits required to enable the nucleic acid to form a precipitate upon the addition of the alcohol:

> The protein denaturing agent is added so as to give a concentration capable of denaturing the protein in the sample at the time when it is added to the sample, and such that thereafter the nucleic acid forms a precipitate on addition of the alcohol. [p. 5].

Thereafter the specification of JP 03101688 describes parameters for various protein denaturing agents (pp. 5-6). The specification also states:

> By means of the above operations, the **nucleic acid** contained in the sample forms a **precipitate**, whereas the **protein** is present **in the solution** in the dissolved state owing to the action of the protein denaturing agent. Consequently, after the method of the present invention has been implemented it is possible to extract or remove the nucleic acid for example by performing a centrifugal separation or membrane separation. [pp. 6-7, emphasis added].

Clearly, JP 03101688 is directed toward the isolation of **precipitated nucleic acids** from **"dissolved" proteins** and, **in contrast to** the present invention, is **not** concerned with the separation of **one type of nucleic acid in one phase** from **another type of nucleic acid in a different phase**.

As noted previously, the example in the JP03101688 abstract never states whether the plasmid DNA from the *E. coli* culture medium is isolated from the genomic *E. coli* DNA and how this isolation is achieved, nor does the abstract raise the issue in any way. In fact, the abstract describes the separation of "nucleic acids...from samples" and **does not even suggest** that the **separation of different types of DNA** from each other would be **desirable**. Moreover, the specification of JP03101688 likewise never addresses the separation of different types of DNA. Likewise, as noted previously, Practical Example 3 (p. 9), which describes the above isolation of DNA from the plasmid-transformed *E. coli* strain, merely focuses on whether the resulting DNA can be subjected to various restriction enzyme and polymerase reactions without inhibition and contains no discussion of genomic DNA. Practical Examples 1, 2, and 4 deal with human cancer cells and make no mention of plasmids. Practical Example 5 uses M13 phage. Claim 1 provides methods for selective extraction of plasmid DNA. Thus, JP03101688 did not anticipate, or suggest, the invention of claim 1.

Claims 2-3, 5, 6, and 38 are dependent on claim 1. The arguments outlined *supra* for claim 1 likewise apply to these dependent claims.

In addition to the above arguments, claim 2 claims the "method of claim 1, wherein the conditions to denature the genomic DNA comprise basic conditions or a temperature of at least 65°C," and claim 3 (amended) is further limited to "basic conditions in which a base is present." The JP 03101688 abstract never addresses the issue of temperature for denaturation. As noted *supra*, the abstract also **never addresses** the issue of denaturation of **genomic DNA - only** the denaturation of **proteins**.

Claim 24 currently reads as follows:

> 24 (Twice amended). An extraction mixture for selectively extracting plasmid DNA from genomic DNA in a DNA-containing material which comprises plasmid DNA and genomic DNA, which extraction mixture comprises butanol, a chaotrope, and water.

Claims 28, 32, and 33 are dependent on claim 24 or upon a claim dependent on claim 24.

Again, as noted previously, the JP 03101688 abstract describes the separation of **nucleic acids** from **samples or cultures - not** an extraction mixture for **selectively** extracting **plasmid DNA** from a **DNA containing material** which comprises plasmid DNA and **genomic DNA**, as provided in claim 24. The abstract **never even suggests** that isolation of one type of DNA from another would be desirable. The specification likewise neither teaches nor suggests these features, as discussed *supra*. The arguments that apply to claim 24 likewise apply to dependent claims 28, 32, and 33.

In view of the foregoing remarks, Applicants respectfully assert that the present claims were not anticipated, or would not have been suggested, by JP 03101688. Therefore, Applicants respectfully request reconsideration and withdrawal of the rejections made under 35 U.S.C. 102(b).

IV. Rejection of Claims 1-12, 21-29, 32, 33, 36, and 38 Under 35 U.S.C. § 102(b), or, in the Alternative, Under 35 U.S.C. § 103(a), Is Traversed

In the Office Action at page 3 (paragraph 10), the Examiner has rejected claims 1-12, 21-29, 32, 33, 36, and 38 as being anticipated by, or in the alternative, obvious over, JP 03101688. This rejection is respectfully traversed. The Examiner states:

> JP 03101688 taught (see the abstract) a method of isolating plasmid DNA from an aqueous mixture of plasmid DNA and genomic DNA by adding a chaotrope (guanidinium salt) and butanol, then isolating the plasmid. The aqueous solution was at a basic pH.
>
> JP 03101688 did not explicitly teach that the basic pH of the solution was produced by adding sodium hydroxide. However, sodium hydroxide is a notorious and well known basic reagent used to produce a basic pH in a solution containing plasmid DNA and genomic DNA as shown in WO 99/61603 (see especially page 12).
>
> The instant specification teaches that n-butanol, 2 methylpropanol and butan-2-ol are obvious and well known forms of butanol, which would therefore be obvious to one of ordinary skill in the art at the time of filing the instant application, absent any unexpected results. [Office Action, paragraph 10].

Claims 1-3, 5, 6, 24, 28, 32, 33, and 38 have been described, *supra*. Claims 4, 7-12, 21-23 and 36 are dependent on claim 1 or upon a claim dependent on claim 1. Claims 25-27 and 29 are dependent on claim 24 or upon a claim dependent on claim 24.

Claim 4 claims the method of claim 1, "wherein the butanol is n-butanol, 2-methylpropanol, or butan-2-ol." Claim 7 claims the method of claim 3, "wherein the base comprises a hydroxide." Claim 8 claims the method of claim 7, "wherein the hydroxide comprises sodium hydroxide." Claim 9 claims the method of claim 3, "wherein the butanol, the chaotrope, the base and the water are combined to form an extraction mixture, and extraction step (i) comprises mixing the extraction mixture with the plasmid DNA-containing material." Claim 10 claims the method of claim 1, "wherein the amount of butanol is in the

range from 20 to 70% based on the volume of the combination of butanol, chaotrope and water." Claim 11 claims the method of claim 10, "wherein the amount of the butanol is in the range from 35 to 50%." Claim 12 claims the method of claim 11, "wherein the amount of the butanol is about 42%." Claim 21 claims the method of claim 1, "which further comprises a step of separating the butanol and aqueous phases of step (i) prior to recovering the plasmid DNA." Claim 22 claims the method of claim 21, "wherein the step of separating the butanol and aqueous phases further comprises centrifugation of the mixture formed in step (i) to facilitate separation of the mixture into the butanol and aqueous phases." Claim 23 claims the method of claim 1, "wherein the DNA-containing material comprises a lysed or unlysed bacterial culture." Claim 36 claims the method of claim 1, "wherein the conditions of extracting step (i) further comprise forming an aqueous phase and a butanol phase."

Claim 25 claims the "extraction mixture of claim 24, which further comprises a base." Claim 26 claims the extraction mixture of claim 25, "wherein the base comprises a hydroxide." Claim 27 claims the mixture of claim 26, "wherein the hydroxide comprises sodium hydroxide." Claim 29 claims the mixture of claim 24, "wherein the butanol constitutes from 20 to 70% based on the volume of the extraction mixture."

WO 99/61603 describes a buffer, a kit and methods for "separating and/or isolating circular nucleic acids from a mixture having different species of nucleic acids other than circular nucleic acids" under alkaline conditions (pH > 8) with a solid matrix of silica material and "at least one chaotropic substance" (claim 1 and throughout). The specification describes the making of mixtures of linear and circular nucleic acids, which are then bound to silica material and the selective washing away of linear DNA with a chaotropic buffer at alkaline pH (Examples 1-3; pp. 21-23). It also describes the use of multi-step protocols with multiple buffers used to isolate the genomic and plasmid DNA from transformed *E. coli* onto silica membrane, resin or particles (Examples 4-6; pp. 16-21 and 24) for the subsequent removal of the genomic DNA and isolation of the plasmid DNA. The specification describes the observation of Birnboim & Doly (Nucl. Acids Res. 7(6): 1513-1523 (1979)) concerning the finding that at pH 12.0-12.5 plasmid DNA remains undenatured, whereas chromosomal DNA

is denatured. The specification then discusses the combined effects of a chaotrope in shifting this pH effect to lower and broader pH values.

In addition, the specification states:

> Furthermore, the invention takes advantage of the effect that under the conditions - presence of high molar chaotropic substances at alkaline pH - circular closed double stranded DNA (e.g., plasmid) but not linear DNA fragment (e.g., sheared chromosomal DNA) specifically binds to silica material. The precise pH may be adjusted with all kinds of pH buffers effective in this range of pH. Examples are phosphate buffers, glycine buffers, and boric acid/sodium hydroxide buffer. Due to its high buffer capacity in particular glycine buffers seem to be suitable for this purpose. [pp. 11-12].

While WO 99/61603 notes the desirability of isolating plasmid DNA from linear DNA and mentions a boric acid/sodium hydroxide buffer, however, it in no way suggests the separation of genomic and plasmid DNA in **separate phases** simply by using basic conditions to denature genomic DNA. Instead it requires **lengthy isolation** protocols using a number of buffers, followed by **binding to a silica matrix** as a condition for separation.

The present invention provides a simplified method (see, e.g., p. 2, ll. 1-3) for isolating plasmid DNA. As noted in the present application, many previously proposed methods require a series of steps or special apparatus (see generally p. 1), whereas "the present invention provides a 'one step' method which is simple to perform and which requires no specialised laboratory apparatus" (p. 2, ll. 17-19). The present invention requires no silica matrix or lengthy isolation steps prior to the mixing of the DNA containing material with butanol, a chaotrope and water as described. For instance, Examples 1 and 2 of the present application simply describe the pelleting of cells by centrifugation followed by resuspension in TE (pp. 5-8) prior to mixing with butanol, a chaotrope and water.

Applicants respectfully submit that neither the abstract, nor the remainder of JP 03101688, anticipated, or suggested, the invention as claimed in claims 1-12, 21-29, 32, 33, 36, or 38. Applicants have already discussed JP 03101688 at length, and the discussion, supra, also applies to this rejection. In addition, the use of basic conditions in WO 99/61603, even as background information, does not cure the deficiencies of JP 03101688, which neither teaches nor suggests separation of plasmid DNA from genomic DNA, let alone by selective denaturation of the genomic DNA only.

In view of the foregoing remarks, Applicants respectfully assert that the present claims were not anticipated, and would not have been suggested, by JP03101688, either with or without WO 99/61603. Therefore, Applicants respectfully request reconsideration and withdrawal of the rejections made under 35 U.S.C. 102(b).

V. The Examiner's Response to Arguments Is Addressed in Sections III and IV, *Supra*

Applicants have addressed the Examiner's Response to Arguments (pages 4-5, pars. 11-13) in sections III and IV, *supra*.

VI. Objection to Claims 13, 14, 30, 31, 34, 35, and 37 Is Accommodated and Rendered Moot

The Examiner has objected to claims 13, 14, 30, 31, 34, 35, and 37 as being dependent upon a rejected base claim (page 5, par. 14), but has indicated that these claims "would be allowable if rewritten in independent form including all of the limitations of the base claim and any intervening claims."

Applicants have amended claims 13, 14, 30, 31, 34, 35, and 37 to accommodate the Examiner's suggestions. Applicants respectfully submit that these claims are now in a condition for allowance and request the Examiner to allow these claims accordingly.

VI. Conclusion

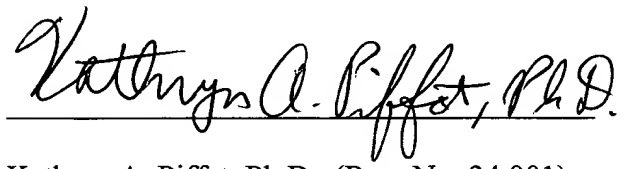
It is believed that all outstanding rejections have been addressed by this submission and that all the claims are in condition for allowance. If discussion of any amendment or remark made herein would advance this important case to allowance, the Examiner is invited to call the undersigned as soon as convenient.

In view of the foregoing amendments and remarks, the present application is respectfully considered in condition for allowance. An early reconsideration and notice of allowance are earnestly solicited.

Applicants hereby request a one-month extension of time for the Amendment and accompanying materials. Although it is not believed that any additional fee (in addition to the fee concurrently submitted) is required to consider this submission, the Commissioner is hereby authorized to charge our deposit account no. 04-1105 should any fee be deemed necessary.

Respectfully submitted,

Date: March 14, 2003



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APPENDIX I

REVISIONS OF CLAIMS PURSUANT TO REVISED RULE § 1.121

Pursuant to Revised Rule § 1.121(c)(1)(ii), the revisions of claims 1, 13-15, 21, 22, 24, 30, 31, 34, 35, and 37 are detailed as follows (marked new claims not required):

1 (Once amended). A method for isolating plasmid DNA from genomic DNA in a DNA containing material which comprises plasmid DNA and genomic DNA, comprising the steps of:

(i) extracting the plasmid DNA into butanol by mixing the material with butanol, a chaotrope, and water under conditions to denature the genomic DNA and forming an aqueous phase and a butanol phase, wherein the genomic DNA is substantially in the aqueous phase and the plasmid DNA is substantially in the butanol phase; and

(ii) recovering the plasmid DNA from the butanol.

13 (Twice amended). A method for isolating plasmid DNA from a DNA containing material which comprises plasmid DNA and genomic DNA, comprising the steps of:

i. extracting the plasmid DNA into butanol by mixing the material with butanol, a chaotrope, and water under conditions to denature the genomic DNA, [The method of claim 1,] wherein the chaotrope is present at a concentration of from 0.7M to 1.2M based on the combination of butanol, chaotrope and water; and

ii. recovering the plasmid DNA from the butanol.

14 (Once amended). A method for isolating plasmid DNA from a DNA containing material which comprises plasmid DNA and genomic DNA, comprising the steps of:

i. extracting the plasmid DNA into butanol by mixing the material with butanol, a chaotrope, and water under conditions to denature the genomic DNA, [The method of claim 13,] wherein the concentration of the chaotrope is about 0.9M; and

ii. recovering the plasmid DNA from the butanol.

15 (Twice amended). The method of claim 1, wherein the recovery step (ii) comprises mixing the [DNA-containing] butanol phase, which comprises plasmid DNA, with a precipitating agent that can precipitate the plasmid DNA from the butanol, and separating the precipitated plasmid DNA from the butanol.

21 (Twice amended). The method of claim 1, which further comprises a step of separating the butanol [organic] and aqueous phases of step (i) prior to recovering the plasmid DNA.

22 (Once amended). The method of claim 21, wherein the step of separating the butanol [organic] and aqueous phases further comprises centrifugation of the mixture formed in step (i) to facilitate separation of the mixture into the butanol [organic] and aqueous phases.

24 (Twice amended). An extraction mixture for selectively extracting plasmid DNA from genomic DNA in a DNA-containing material which comprises plasmid DNA and genomic DNA, which extraction mixture comprises butanol, a chaotrope, and water.

30 (Once amended). An extraction mixture for selectively extracting plasmid DNA from a DNA-containing material which comprises plasmid DNA and genomic DNA, which extraction mixture comprises butanol, a chaotrope, and water, [The extraction mixture of claim 29,] wherein the butanol constitutes from 35 to 50 % of the extraction mixture.

31 (Once amended). An extraction mixture for selectively extracting plasmid DNA from a DNA-containing material which comprises plasmid DNA and genomic DNA, which extraction mixture comprises butanol, a chaotrope, and water, [The extraction mixture of claim 30,] wherein the butanol constitutes about 42% of the extraction mixture.

34 (Twice amended). An extraction mixture for selectively extracting plasmid DNA from a DNA-containing material which comprises plasmid DNA and genomic DNA, which

extraction mixture comprises butanol, a chaotrope, and water, [The extraction mixture of claim 24,] wherein the concentration of chaotrope in the extraction mixture is from 0.7M to 1.2M.

35 (Once amended). An extraction mixture for selectively extracting plasmid DNA from a DNA-containing material which comprises plasmid DNA and genomic DNA, which extraction mixture comprises butanol, a chaotrope, and water, [The extraction mixture of claim 34,] wherein the concentration of the chaotrope in the extraction mixture is about 0.9M.

37 (Once amended). A method for isolating plasmid DNA from genomic DNA in a DNA containing material which comprises plasmid DNA and genomic DNA, comprising the steps of:

i. extracting the plasmid DNA into butanol by mixing the material with butanol, a chaotrope, and water under conditions to denature the genomic DNA and forming an aqueous phase and a butanol phase, [The method of claim 36,]wherein the genomic DNA is substantially in the aqueous phase and the plasmid DNA is substantially in the butanol phase; and

ii. recovering the plasmid DNA from the butanol phase.